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CLAIMS

1. A substantially pure polypeptide fragment which

a) comprises an amino acid sequence selected from the sequences shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 17-23, 42, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72-86, 88, 90, 92, 94, 141, 143, 145, 147, 149, 151, 153, and 168-171,

b) comprises a subsequence of the polypeptide fragment defined in a) which has a length of at least 6 amino acid residues, said subsequence being immunologically equivalent to the polypeptide defined in a) with respect to the ability of evoking a protective immune response against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex, or

c) comprises an amino acid sequence having a sequence identity with the polypeptide defined in a) or the subsequence defined in b) of at least 70% and at the same time being immunologically equivalent to the polypeptide defined in a) with respect to the ability of evoking a protective immune response against infections with mycobacteria belonging to the tuberculosis complex or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with antigens derived from mycobacteria belonging to the tuberculosis complex,

with the proviso that

i) the polypeptide fragment is in essentially pure form when consisting of the amino acid sequence 1-96 of SEQ ID NO: 2 or when consisting of the amino acid sequence 87-108 of SEQ ID NO: 4 fused to β-galactosidase,

ii) the degree of sequence identity in c) is at least 95% when the polypeptide comprises a homologue of a polypeptide which has the amino acid sequence SEQ ID NO: 12 or a subsequence thereof as defined in b), and

iii) the polypeptide fragment contains a threonine residue corresponding to position 213 in SEQ ID NO: 42 when comprising an amino acid sequence of at least 6 amino acids in SEQ ID NO: 42.

2. The polypeptide fragment according to claim 1 in essentially pure form.

3. The polypeptide fragment according to claim 1 or 2, which comprises an epitope for a T-helper cell.

4. The polypeptide fragment according to any of the preceding claims, which has a length of at least 7 amino acid residues, such as at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, and at least 30 amino acid residues.

5. The polypeptide fragment according to any of the preceding claims, which is free from amino acid residues -30 to -1 in SEQ ID NO: 6 and/or -32 to -1 in SEQ ID NO: 10 and/or -8 to -1 in SEQ ID NO: 12 and/or -32 to -1 in SEQ ID NO: 14 and/or -33 to -1 in SEQ ID NO: 42 and/or -38 to -1 in SEQ ID NO: 52 and/or -33 to -1 in SEQ ID NO: 56 and/or -56 to -1 in SEQ ID NO: 58 and/or -28 to -1 in SEQ ID NO: 151.

6. The polypeptide fragment according to any of the preceding claims which is free from any signal sequence.

7. The polypeptide fragment according to any of the preceding claims which

1) induces a release of IFN-γ from primed memory T-lymphocytes withdrawn from a mouse within 2 weeks of primary infection or within 4 days after the mouse has been re-challenge infected with mycobacteria belonging to the tuberculosis complex, the induction performed by the addition of the polypeptide to a suspension comprising about 200.000 spleen cells per ml, the addition of the polypeptide resulting in a concentration of 1-4 μg polypeptide per ml suspension, the release of IFN-γ being assessable by determination of IFN-γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension, and/or

2) induces a release of IFN-γ of at least 300 pg above background level from about 1000,000 human PBMC (peripheral blood mononuclear cells) per ml isolated from TB patients in the first phase of infection, or from healthy BCG vaccinated donors, or from healthy contacts to TB patients, the induction being performed by the addition of the polypeptide to a suspension comprising the about 1,000,000 PBMC per ml, the addition of the polypeptide resulting in a concentration of 1-4 μg polypeptide per ml suspension, the release of IFN-γ being assessable by determination of IFN-γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension; and/or

3) induces an IFN-γ release from bovine PBMC derived from animals previously sensitized with mycobacteria belonging to the tuberculosis complex, said release being at least two times the release observed from bovine PBMC derived from animals not previously sensitized with mycobacteria belonging to the tuberculosis complex.

8. A polypeptide fragment according to any of the preceding claims, wherein the sequence identity in c) is at least 80%, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, and at least 99.5%.

9. A fusion polypeptide comprising at least one polypeptide fragment according to any of the preceding claims and at least one fusion partner.

10. A fusion polypeptide according to claim 56, wherein the fusion partner is selected from the group consisting of a polypeptide fragment as defined in any of claims 1-8, and an other polypeptide fragment derived from a bacterium belonging to the tuberculosis complex, such as ESAT-6 or at least one T-cell epitope thereof, MPB64 or at least one T-cell epitope thereof, MPT64 or at least one T-cell epitope thereof, and MPB59 or at least one T-cell epitope thereof.

11. A fusion polypeptide fragment which comprises

1) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein ESAT-6, and a second amino acid sequence including at least one T-cell epitope derived from a *M. tuberculosis* protein different from ESAT-6 and/or including a stretch of amino acids which protects the first amino acid sequence from *in vivo* degradation or post-translational processing; or

2) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the *M. tuberculosis* protein MPT59, and a second amino acid sequence including at least one T-cell epitope derived from a *M. tuberculosis* protein different from MPT59 and/or including a stretch of amino acids which protects the first amino acid

sequence from *in vivo* degradation or post-translational processing.

12. A fusion polypeptide fragment according to claim 11, wherein the first amino acid sequence is situated C-terminally to the second amino acid sequence.

13. A fusion polypeptide fragment according to claim 11, wherein the first amino acid sequence is situated N-terminally to the second amino acid sequence.

14. A fusion polypeptide fragment according to any of claims 11-13, wherein the at least one T-cell epitope included in the second amino acid sequence is derived from a *M. tuberculosis* polypeptide selected from the group consisting of a polypeptide fragment according to any of claims 1-55, DnaK, GroEL, urease, glutamine synthetase, the proline rich complex, L-alanine dehydrogenase, phosphate binding protein, Ag 85 complex, HBHA (heparin binding hemagglutinin), MPT51, MPT64, superoxide dismutase, 19 kDa lipoprotein, α-crystallin, GroES, MPT59 when the first T-cell epitope is derived from ESAT-6, and ESAT-6 when the first T-cell epitope is derived from MPT59.

15. A fusion polypeptide fragment according to any of claims 11-14, wherein the first and second T-cell epitopes each have a sequence identity of at least 70% with the natively occurring sequence in the proteins from which they are derived.

16. A fusion polypeptide according to any of claims 11-15, wherein the first and/or second amino acid sequence have a sequence identity of at least 70% with the protein from which they are derived.

17. A fusion polypeptide fragment according to any of claims 11-16, wherein the first amino acid sequence is the amino acid sequence of ESAT-6 or of MPT59 and/or the second amino acid sequence is the amino acid sequence of a *M. tuberculosis*

polypeptide selected from the group consisting of a polypeptide fragment according to any of claims 1-8, DnaK, GroEL, urease, glutamine synthetase, the proline rich complex, L-alanine dehydrogenase, phosphate binding protein, Ag 85 complex, HBHA (heparin binding hemagglutinin), MPT51, MPT64, superoxide dismutase, 19 kDa lipoprotein, α-crystallin, GroES, ESAT-6 when the first amino acid sequence is that of MPT59, and MPT59 when the first amino acid sequence is that of ESAT-6.

18. A fusion polypeptide fragment according to any of claims 11-17, which comprises ESAT-6 fused to MPT59.

19. A fusion polypeptide fragment according to claim 18, wherein no linkers are introduced between the two amino acid sequences.

20. A polypeptide according to any of the preceding claims which is lipidated so as to allow a self-adjuvating effect of the polypeptide.

21. A substantially pure polypeptide according to any of claims 1-20 for use as a pharmaceutical.

22. The use of a substantially pure polypeptide according to any of claims 1-20 in the preparation of a pharmaceutical composition for the diagnosis of or vaccination against tuberculosis caused by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

23. A nucleic acid fragment in isolated form which

1) comprises a nucleic acid sequence which encodes a polypeptide as defined in any of claims 1-20, or comprises a nucleic acid sequence complementary thereto,

2) has a length of at least 10 nucleotides and hybridizes readily under stringent hybridization conditions with a

fragment contains an A corresponding to position 781 in SEQ ID NO: 41 and when the nucleic acid fragment comprises a subsequence of a nucleotide sequence exactly complementary to SEQ ID NO: 41, then the nucleic acid fragment comprises a T corresponding to position 781 in SEQ ID NO: 41.

24. A nucleic acid fragment according to claim 23, which is a DNA fragment.

25. A vaccine comprising a nucleic acid fragment according to claim 23 or 24, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer substantially increased resistance to infections with mycobacteria of the tuberculosis complex in an animal, including a human being.

26. A nucleic acid fragment according to claim 23 or 24 for use as a pharmaceutical.

27. The use of a nucleic acid fragment according to claim 23 or 24 in the preparation of a pharmaceutical composition for the diagnosis of or vaccination against tuberculosis caused by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*.

28. An immunologic composition comprising a polypeptide according to any of claims 1-20.

29. An immunologic composition according to claim 28, which further comprises an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.

30. An immunologic composition according to claim 29, wherein the carrier is selected from the group consisting of a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction, such as a plastic, e.g. polystyrene, a polymer to which the polypeptide(s) is/are covalently

bound, such as a polysaccharide, and a polypeptide, e.g. bovine serum albumin, ovalbumin or keyhole limpet hemocyanin; the vehicle is selected from the group consisting of a diluent and a suspending agent; and the adjuvant is selected from the group consisting of dimethyldioctadecylammonium bromide (DDA), Quil A, poly I:C, Freund's incomplete adjuvant, IFN-γ, IL-2, IL-12, monophosphoryl lipid A (MPL), and muramyl dipeptide (MDP).

31. An immunologic composition according to any of claims 28 to 30, comprising at least two different polypeptide fragments, each different polypeptide fragment being a polypeptide according to any of claims 1-67.

32. An immunologic composition according to claim 78, comprising 3-20 different polypeptide fragments, each different polypeptide fragment being according to any of claims 1-20.

33. An immunologic composition according to any of claims 28-32, which is in the form of a vaccine.

34. An immunologic composition according to any of claims 28-32, which is in the form of a skin test reagent.

35. A vaccine for immunizing an animal, including a human being, against tuberculosis caused by mycobacteria belonging to the tuberculosis complex, comprising as the effective component a non-pathogenic microorganism, wherein at least one copy of a DNA fragment comprising a DNA sequence encoding a polypeptide according to any of claims 1-20 has been incorporated into the genome of the microorganism in a manner allowing the microorganism to express and optionally secrete the polypeptide.

36. A vaccine according to claim 35, wherein the microorganism is a bacterium.

37. A vaccine according to claim 36, wherein the bacterium is selected from the group consisting of the genera *Mycobacterium*, *Salmonella*, *Pseudomonas* and *Eschericia*.

38. A vaccine according to claim 37, wherein the microorganism is *Mycobacterium bovis* BCG, such as *Mycobacterium bovis* BCG strain: Danish 1331.

39. A vaccine according to any of claims 35-38, wherein at least 2 copies of a DNA fragment encoding a polypeptide according to any of claims 1-20 are incorporated into the genome of the microorganism.

40. A vaccine according to claim 39, wherein the number of copies is at least 5.

41. A replicable expression vector which comprises a nucleic acid fragment according to claim 23 or 24.

42. A vector according to claim 41, which is selected from the group consisting of a virus, a bacteriophage, a plasmid, a cosmid, and a microchromosome.

43. A transformed cell harbouring at least one vector according to claim 41 or 42.

44. A transformed cell according to claim 43, which is a bacterium belonging to the tuberculosis complex, such as a *M. tuberculosis bovis* BCG cell.

45. A transformed cell according to claim 43 or 44, which expresses a polypeptide according to any of claims 1-20.

46. A method for producing a polypeptide according to any of claims 1-20, comprising

inserting a nucleic acid fragment according to claim 23 or 24 into a vector which is able to replicate in a host cell,

introducing the resulting recombinant vector into the host cell, culturing the host cell in a culture medium under conditions sufficient to effect expression of the polypeptide, and recovering the polypeptide from the host cell or culture medium; or

isolating the polypeptide from a short-term culture filtrate as defined in claim 1; or

isolating the polypeptide from whole mycobacteria of the tuberculosis complex or from lysates or fractions thereof, e.g. cell wall containing fractions; or

synthesizing the polypeptide by solid or liquid phase peptide synthesis.

47. A method for producing an immunologic composition according to any of claims 28-32 comprising

preparing, synthesizing or isolating a polypeptide according to any of claims 1-20, and

solubilizing or dispersing the polypeptide in a medium for a vaccine, and

optionally adding other *M. tuberculosis* antigens and/or a carrier, vehicle and/or adjuvant substance,

or

cultivating a cell according to any of claims 37-45, and

transferring the cells to a medium for a vaccine, and

optionally adding a carrier, vehicle and/or adjuvant substance.

48. A method of diagnosing tuberculosis caused by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis* in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide according to any of claims 1-20 or an immunologic composition according to claim 34, a positive skin response at the location of injection being indicative of the animal having tuberculosis, and a negative skin response at the location of injection being indicative of the animal not having tuberculosis.

49. A method for immunising an animal, including a human being, against tuberculosis caused by mycobacteria belonging to the tuberculosis complex, comprising administering to the animal the polypeptide according to any of claims 1-20, the immunologic composition according to claim 33, or the vaccine according to any of claims 35-40.

50. A method according to claim 49, wherein the polypeptide, immunologic composition, or vaccine is administered by the parenteral (such as intravenous and intraarterially), intraperitoneal, intramuscular, subcutaneous, intradermal, oral, buccal, sublingual, nasal, rectal or transdermal route.

51. A method for diagnosing ongoing or previous sensitization in an animal or a human being with bacteria belonging to the tuberculosis complex, the method comprising providing a blood sample from the animal or human being, and contacting the sample from the animal with the polypeptide according to any of claims 1-20, a significant release into the extracellular phase of at least one cytokine by mononuclear cells in the blood sample being indicative of the animal being sensitized.

52. A composition for diagnosing tuberculosis in an animal, including a human being, comprising a polypeptide according to any of claims 1-20, or a nucleic acid fragment according to claim 23 or 24, optionally in combination with a means for detection.

53. A monoclonal or polyclonal antibody, which is specifically reacting with a polypeptide according to any of claims 1-20 in an immuno assay, or a specific binding fragment of said antibody.